Ver. HB230112

Anti-DYKDDDDK (Flag) Affinity Gel

Product description

Anti-DYKDDDDK (Flag) Affinity Gel is prepared by conjugating high quality murine IgG2b monoclonal antibody with Sepharose 4B gel. This product has a high Flag label fusion protein loading capacity (at least 1.1 mg protein/mL gel) and less non-specific binding of miscellaneous proteins. It can be used for immunoprecipitation (IP) and purification of fusion proteins with Flag labels.

Anti-DYKDDDDK (FLAG) Affinity Gel can bind to the Met modified N-flag fusion Protein (MET-flag-Protein), N-flag fusion protein (Flag-protein), C-terminal FLAG Fusion Protein (protein-flag).

Components

Components No.	Name	20585ES01	20585ES03	20585ES08	20585ES25	20585ES60
20585	Anti-DYKDDDDK (Flag) Affinity Gel	100 μL	1 mL	5 mL	25 mL	100 mL

Specifications

Clone	1E6
Isotype	Mouse IgG2b
Purity	Protein A
Antibody Concentration	7.5 g antibody/L Gel
Application	Protein purification、IP
Protein binding capacity	⩾1.1 mg protein/mL Gel
Storage buffer	10 mM Na_3PO_4,150,mM NaCl，50% glycerin，pH 7.4，contains 0.02% (w/v) sodium azide

Storage

The unopened products should be stably stored at -25°C ~-15°C for 1 year. Do not freeze in solution without glycerin!

Instructions

1. **Preparation of cell lysate (Taking mammalian cells as an example)**

Note: The sample should not contain insoluble particles, which can be filtered by 0.22 μm filter membrane or centrifuged by 10,000×g for 15 min. Protease inhibitors can be added if necessary; If the sample is too sticky, it can be treated with nuclease.

Cell density 70-90%, 100 mm petri dish (about 10^6-10^7 cells), adding 1mL lysate (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1%Triton X-100). A protease inhibitor Cocktail (Cat 20123ES) was recommended.

1.1 Wash the cells

Adherent cells: Growth medium was removed, washed twice with PBS, PBS was removed, and lysate (10^6-10^7 cells/mL) was added.

Suspension cells: The cells were transferred to a centrifuge tube, centrifuged at 420×g for 5 min, and the supernatant was removed. Re-suspension was performed with PBS, centrifuged at 420×g for 5 min, repeated once, supernatant was removed, and lysis solution was added for re-suspension (10^6-10^7 cells/mL).

1.2 After adding the lysate, incubate for 15-30 min.

1.3 Centrifugation, 12,000×g, 10 min. Scrape off the adherent cells before centrifugation.

1.4 Collect the supernatant into the pre-cooled sample tube. Supernatant can be stored at -70°C.

2. Application

The target protein can be purified using chromatographic columns or immunoprecipitation (IP). About 100 mL of cell lysate can be purified by 1-3 mL gel chromatography column. If the sample volume is small (1-2 mL of cell lysate), immunoprecipitation (IP) can be used. Solution systems are recommended for handling large volumes of cell lysate.

2.1 Chromatographic column method

2.1.1 Column Installation

1) Prepare the empty chromatographic column (Cat 20520ES-20526ES); Wash the column twice with TBS (50 mM Tris-HCl, pH 7.4,150 mM NaCl) or other suitable buffer solution that is 2-3 times the column volume.

2) Reverse mix Anti-Flag affinity purification gel to ensure gel uniformity. Take 1-3 mL gel packed column (purified about 100 mL cell lysate).

3) Wash twice with TBS of 2-3 times the volume of the column. After the liquid runs out, rinse with 0.1 M Gly-HCl,pH 3.5 of 3 times the volume to ensure the smooth gel surface. Gly-HCl buffer treatment time should not exceed 20 min.

4) Balance the gel column with 5 times the volume of TBS until the effluent reaches a neutral pH.

2.1.2 Purification

1) Loading: Fill the chromatographic column with protein cracking solution and repeat loading 2-3 times to increase the binding amount of protein and gel as much as possible.

2) Cleaning: Cleaning with 10-20 times the column volume of TBS to remove the specific binding proteins.

3) Elution (one of the following two methods can be selected) :

A. Acid elution: 15-25 μL 1 M Tris, pH 8.0 were added into the collection tube, then 1 mL 0.1 M Gly-HCl, pH 3.5 was used to elute. Repeat for 5 times.

B. 3×Flag polypeptide (Cat#20571ES) Elution: Flag polypeptide solution (100 μg/mL) prepared with TBS was eluted, and eluted with one-column volume polypeptide solution, respectively, four times.

2.1.3 Regeneration and preservation of filler

1) Column regeneration: regeneration immediately after use, clean with 0.1M Gly-HCl,pH3.5(3 times column volume), immediately balance with TBS until neutral pH is reached.

2) Preservation: Clean with 10 times the volume of the column TBS (containing 50% glycerin and 0.02% sodium azide), then add 5 mL of the solution to the column, and store at 2-8°C or -20°C.

2.2 Immunoprecipitation (IP)

2.2.1 Immunoprecipitation (IP)

1) Fully re-suspension of Anti-Flag affinity purification gel to form a uniform solution as far as possible. Put 40 μL of the mixture (20 μL gel) into the new centrifuge tube.

2) Centrifuge at 5,000-8,200×g for 30 s to precipitate the gel at the bottom of the centrifuge tube, and let it stand for 1-2 min before adding the sample. Remove supernatant, carefully at this point to avoid absorbing gel.

3) Add 500 μL TBS, gently reinsert the Anti-Flag Affinity Gel, centrifuge at 10,000 rpm for 30 s, discard the supernatant, and repeat the above steps once.

4) Optional steps. In order to remove the unbound antibodies in the gel, 500μL 0.1M glycine HCl, pH 3.5 was added to clean the gel, and supernatant was removed by centrifugation. TBS of 3 times the volume was added. Then Shake gently for 2-3 min and centrifuge at 5,000-8,200×g for 30 s. The supernatant was discarded, and the pH of the last clear was neutral after repeated washing. The treatment time of Glycine HCl should not exceed 20 min.

5) Add 200-1000 μL cell lysate, and adjust the lysate to the final volume of 1 mL if necessary. The volume of cell lysate depends on the expression of Flag fusion protein. For positive control, 1 mL TBS and 4 μL 50 ng/μL Flag-BAP fusion protein (about 200 ng) were added. For negative control, just add 1 mL cleavage buffer (without protein).

6) Incubate slowly at 4°C for 2 hours. If you need to improve the binding efficiency, can be extended to overnight.

7) Centrifuge at 5,000-8,200×g for 30 s to remove supernatant.

8) The above precipitation was lightly mixed with 0.5mL TBS, centrifugated at 5,000-8,200×g for 30 s to exhaust the supernatant, and repeated 3 times.

2.2.2 Elution of Flag Fusion Protein (one of the following three methods can be selected)

1) Non-denatured elution (3×Flag polypeptide)

A. Preparation of 3×Flag polypeptide eluent: The 3×Flag polypeptide was dissolved in 0.5 M Tris-HCl solution at pH 7.5 (containing 1M NaCl), and the final concentration was 25 μg/μL. Diluted to 5 μg/μL with ddH_2O, 3 μL 5 μg/μL 3×Flag polypeptide was added to 100 μL TBS (final concentration 150 ng/μL).

B. Add 100 μL 3×Flag polypeptide eluent, respectively, incubate at 4°C for 30 min (slowly shaking), centrifuge at 5,000-8,200×g for 30 s, and transfer the supernatant to the new tube (do not suck gel). If used immediately, the supernatant can be stored at 4°C, -20°C for a long time.

2) Acid elution (0.1M Gly-HCl, pH 3.5)

Add 100 μL 0.1 M Gly-HCl, pH 3.5, incubate at room temperature for 5 min (slowly shaking), centrifuge at 5,000-8,200×g for 30 s, transfer the supernatant to a new tube (containing 10 μL 0.5 M Tris-HCl, pH 7.5, 1.5 M NaCl). Be careful not to inhale the gel. If used immediately, the supernatant can be stored at 4°C, -20°C for a long time.

3) Elution with SDS-PAGE loading buffer

20 μL 2× loading buffer (125 mM Tris-HCl, pH 6.8, 4% SDS, 20% glycerin, 0.004% bromophenol blue) was added, boiled for 3 min, and centrifugated for 30 s at 5,000-8,200×g. Supernatant can be directly SDS-PAGE electrophoresis, or WB detection.

2.2.3 Regeneration and preservation of filler

1) Regeneration: Immediately after use, add 0.1M Gly-HCl, pH3.5, 3 times the gel volume, shake gently for 3-5 min, centrifuge at 5,000-8,200×g for 30 s, discard the supernatant. Immediately balance with TBS: add 3 times the volume of TBS, gently shake for 2-3 min, centrifuge at 5,000-8,200×g for 30 s, collect supernatant to determine pH, if neutral, then proceed to the next step, if still acidic, repeat the balance step.

2) Preservation: Clean with 10 times gel volume TBS (containing 50% glycerin and 0.02% sodium azide), then add appropriate amount of the preservation solution to the filler, and store at 2-8°C or -20°C. The amount of buffer added can be restored to before purification. The volume ratio of glue: buffer =1:1, which can increase the volume of buffer liquid. The buffer is mainly to provide the necessary pH value and avoid freezing at -20°C.

3 Solution system

3.1 Adjusting the pH of protein lysate to pH 7-8, containing 0.15M salt ions, such as NaCl, can reduce non-specific protein binding. The insoluble components of Flag fusion protein lysate should be removed.

3.2 The Anti-Flag affinity purification gel was re-suspended and transferred to a new centrifuge tube. 3 times the volume of TBS, gently shake for 2-3 min, centrifuge at 5,000-8,200×g for 30 s, discard the supernatant. Repeat washing until the pH is neutral.

3.3 The Flag fusion protein lysate was incubated with Anti-Flag affinity purification gel for 1 h, and shook gently during incubation to ensure full contact between protein and gel. The incubation time can be adjusted according to the situation.

3.4 Centrifuge at 1000 ×g for 5 min to collect gel-Flag fusion protein.

3.5 Wash the gel with 10-20 times the column volume of TBS to remove specific proteins.

3.6 Eluting Flag fusion protein [refer to the above elution steps].

3.7 Regeneration and preservation of filler [refer to the above steps].

Notes

1. During acid elution, the treatment time of Gly-HCl buffer solution should not exceed 20 min.
2. Do not add reducing agents (such as DTT) to the loading buffer, as reducing agents will destroy antibodies. If it is necessary to add reducing agents, the reducing agent should not exceed 10% or 100 mM in the 2×loading buffer.
3. SDS in the loading buffer will destroy antibodies, so Anti-Flag affinity purification gel cannot be reused.
4. For protein purification, it is recommended to pack the gel into a chromatographic column where all the solution can flow directly through. If in the tube, need to repeatedly centrifuge to remove the solution, the operation is tedious.
5. Except for samples and cracking solution, other solutions are recommended to be 3 times the volume and repeated 3 times. On the basis of ensuring the purification effect, the number of repeats can be reduced appropriately.
6. Both positive and negative control groups are recommended.
7. For your safety and health, please wear lab coats and disposable gloves for operation.
8. For research use only.